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A Rapid Bioassay Using the Green Alga *Selenastrum capricornutum* to screen for toxicity in St. Lawrence River Sediment Elutriates

by

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ABSTRACT

A toxicty bioassay using the green alga *Selenastrum capricornutum* and measuring inhibition of photosynthetic $^{14}CO_2$ assimilation is described. The essential test specifications are: experimental vessel: 16-mL test tubes; exposure and incubation times: 20 + 4 h, dilution water: demineralized H_2O; algal cell density: 1.9×10^5 cells mL^{-1}: radioactivity: 0.072 µCi mL^{-1}, as $Na_2(H^{14}CO_3)_2$; nutrient enrichment: 1 mL of 10X PAAP medium; and, isotope partitioning: acidification and bubbling. The resulting method is rapid, sensitive, and reliable (cv < 10%) and is applied to elutriates of St-Lawrence River sediments in an ongoing research project.

KEY WORDS: Toxicity, bioassay, algae, *Selenastrum capricornutum*, sediments, elutriates, $^{14}CO_2$-uptake, St. Lawrence River, $CuCl_2$.

INTRODUCTION

The green alga *Selenastrum capricornutum* Printz (Chlorophyta; Chlorophyceae; Chlorococcales) became a widely used bioassay organism with publication of the Provisional Algal Assay Procedure (PAAP) [1] and the Algal Assay Procedure Bottle Test (AAPBT) [2, 3]. Early experiments with this alga were oriented towards measurement of the effects of nutrient additions [4-11] , but its value for toxicity assessment was soon recognized [12-32], and *S. capricornutum* has now become a standard tool in aquatic ecotoxicology. Most researchers have retained the use of batch cultures in bottles as their experimental units, but some recent efforts at miniaturization have appeared [33-34].

The standard bioassay measures growth of the culture, through direct counts of cell numbers [16, 22], automated particle counting [19, 21, 23, 24, 28, 31, 32, 33, 34], or dry-weight biomass estimates [8]. Incubation periods are usually 4d [19, 32, 33], 7d [10, 16, 19], or 8d [23, 31, 33, 34]. *S. capricornutum* has also been used in bioassays measuring inhibition of DCMU-induced fluorescence (a 24-h test) [24], oxygen evolution (1-h procedure) [27], and cellular ATP (4h, 4d, 7d or 8d) [24, 29, 33].

The use of inhibition of photosynthetic uptake of $^{14}CO_2$ as the measure of toxicity in *Selenastrum* tests is a relatively recent development. Such tests have had 20 or 24-h toxicant exposure periods in 100 [30], 125 [24] or 500-mL [26] flasks. The exposure has then been followed by incubation with the radioisotope for periods of 10 min [24], 2 h [26] or 4 h [30].

The present communication is the result of three years of effort to develop a rapid, cost-effective algal bioassay in relatively small vessels for use in screening sediment elutriates

for toxic potential. Special attention has been paid to reducing the variability of the test to an acceptable level.

THE STUDY AREA

In 1984 we began a collaborative project (Environment Canada and l'Université de Montréal) to study the hazard associated with contamination of sediments in the depositional basins of the St. Lawrence River system in the vicinity of Montréal. These basins receive dissolved and suspended loads of contaminants from the Great Lakes-St. Lawrence River Corridor and the Ottawa River basin, as well as locally generated pollutants [35]. Systematic sampling of depositional basins began in May 1984, followed by chemical analysis of sediments [36] and their elutriates [37], and mapping of contaminant distributions [36]. A suite of bioassays is performed on each elutriate.

THE BASIC METHOD

The bioassay involves the calculation of a photosynthesis inhibition curve for each elutriate. The algal culture (*Selenastrum capricornutum* Printz, obtained from the Environmental Protection Service, Longueuil, Québec) is incubated at 26 ± 3 °C with a 12h:12h photoperiod in PAAP medium [38] for 6 days before each bioassay. At the begining of the test, culture is added to experimental vessels at a density of 1.9×10^5 cells mL^{-1}. After an adaptation period, control and treatment vessels receive inoculations of ^{14}C, as $Na_2(H^{14}CO_3)_2$. A series of at least six concentrations of elutriate is used for each test, and results are expressed as percentages of control photosynthesis.

DEVELOPMENTAL OBJECTIVES

We began with a standard ecological technique for measuring algal photosynthesis by $^{14}CO_2$ uptake [39-41]. We converted this to a useful toxicity bioassay by addressing the following objectives: smaller incubator space requirements, reduced sample volumes, lower cost of materials, greater sensitivity to contaminants, and increased statistical reliability.

Space, sample volume, and materials:

By changing our incubation vessels from 128-mL bottles to 16-mL test tubes, we eliminated our incubator space problem and greatly reduced the volume of elutriate needed for our bioassays. We are also able to use small quantities of isotope, adding only 1.15 μCi to each tube, while actually increasing the available isotope per mL of culture volume (0.072 μCi mL^{-1}).

Sensitivity:

The lack of response in some of our preliminary tests led us to suspect that stimulation, due to enrichment from dissolved nutrients in the elutriates, could be masking toxic (inhibitory) effects. Parallel tests using known concentrations of a single toxicant ($CuCl_2$), showed low toxicity and high variability in unenriched tubes (Fig. 1a) relative to a similar test with PAAP algal culture medium [38] added (Fig. 1b). By adding enough nutrient to preclude limitation at all exposure concentrations, toxic responses (inhibition relative to controls) are isolated [24] and the sensitivity of the test as a toxicity bioassay improves. Consequently, we add 1 mL of concentrated (10X) PAAP algal culture medium to each tube.

The period of exposure to the toxic material (adaptation period) before incubation with the radioisotope was also found to be critical. Again using known concentrations of $CuCl_2$, a series of tests was performed with total exposure periods of 4 h (no adaptation phase), 24 h (20 h adaptation) and 48 h (44 h adaptation). The 24-h tests were much more sensitive than the 4-h tests, but a 48-h period yielded no further improvement.

In order to apply the modified test to our study material, St. Lawrence River sediment elutriates, the question of dilution water still had to be considered. Surface waters, even though filtered, may contain dissolved substances capable of complexing or chelating toxicants, thereby altering their observed toxicity [45-46]. In another series of tests we compared the toxicity of elutriates using two diluants, St. Lawrence River water and demineralized water. Toxicity was much higher, and variability much lower, with demineralized water (Fig. 2).

Reliability:

We found filtration to be an unacceptable method of separating assimilated from unassimilated isotope. Filtration frequently took longer than 10 min., which created inefficient backlogs in sample processing flow. Filtration times are highly variable, which may have been the cause of elevated coefficients of variation, as high as 25%. We now use the acidification-bubbling method to drive off unassimilated radiocarbon [43-44]. After the 4-h incubation, a 3-mL subsample is removed from each culture tube and added to a scintillation vial. The subsample is then acidified with 100 µL of 0.1N HCl and bubbled with air for 5 min. 10 mL of a two-phase scintillation cocktail (Aquasol-2™, New England Nuclear) is added to each vial, followed by counting on an LKB-Wallac Rack-Beta™ counter with automatic quench correction. This procedure obviates the need for filtration, saving time and dramatically reducing variability between replicates. The coefficient of variation for our bioassays is now always less than 10%.

CONCLUSIONS

There are several advantages to a photosynthesis bioassay. The test takes only 24 hours to complete. Smaller vessels, and therefore less space, can be used. Finally, experimental units can be easily replicated, giving more statistial power where needed. We are now satisfied that we have a sensitive, reliable bioassay that performs well with single toxicants and equally well with complex, unknown toxicant mixtures (e.g., sediment elutriates). Results of this bioassay on elutriates from St. Lawrence River sediments indicate that sediments from some stations are toxic while others are not. A statistical pre-model, using correlation and multiple regression techniques, showed a significant inhibitory effect by Arsenic in the elutriates from the various stations [42].

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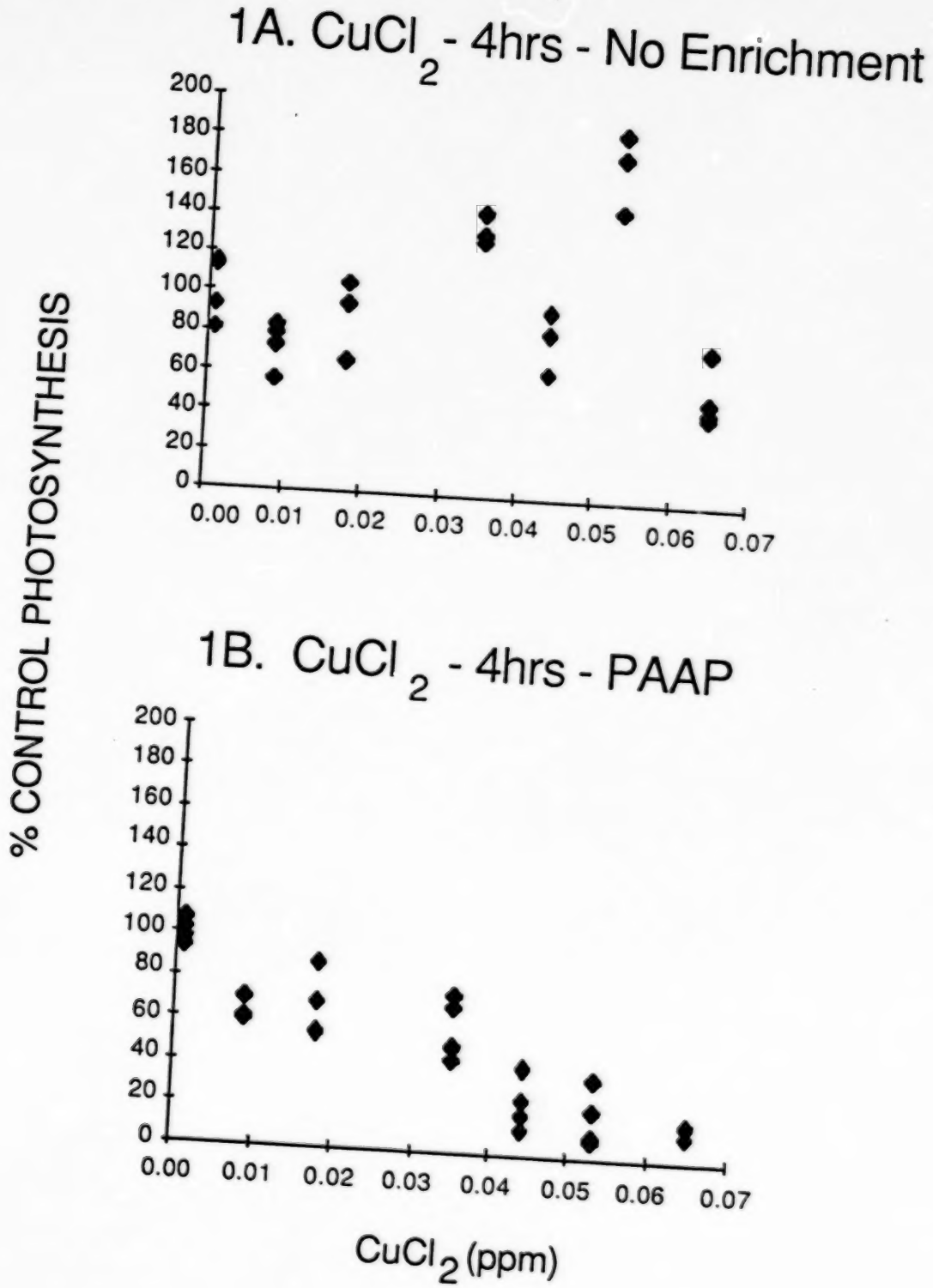
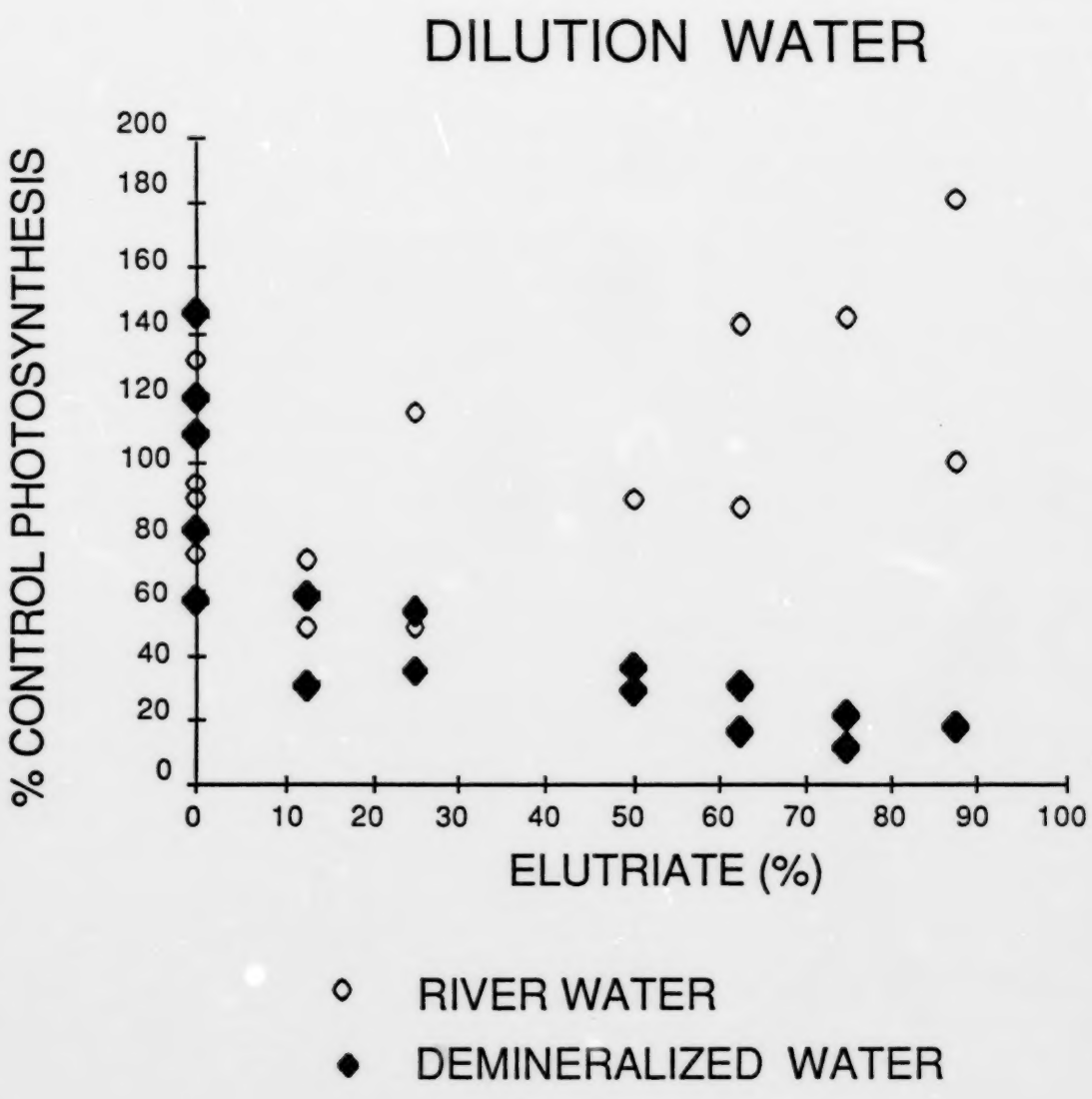
FIGURE LEGENDS

FIGURE 1: Photosynthetic response of *Selenastrum capricornutum* to varying concentrations of $CuCl_2$. 1A: 4 h exposure with no nutrient enrichment. 1B: 4 h exposure, nutrient enrichment with PAAP medium.

FIGURE 2: Photosynthetic response of *Selenastrum capricornutum* to varying concentrations of Lake St. Pierre sediment elutriate. 24 h exposure, nutrient enrichment with PAAP medium. Open symbols: tests with filtered St. Lawrence River water as the diluant. Closed symbols: tests with demineralized water as the diluant.


1A. CuCl2 - 4hrs - No Enrichment
1B. CuCl2 - 4hrs - PAAP
% CONTROL PHOTOSYNTHESIS
CuCl2 (ppm)
0
20
40
60
80
100
120
140
160
180
200
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07


DILUTION WATER
% CONTROL PHOTOSYNTHESIS
200
180
160
140
120
100
80
60
40
20
0
0
10
20
30
40
50
60
70
80
90
100
ELUTRIATE (%)
RIVER WATER
DEMINERALIZED WATER